Compost Tea as a Container Medium Drench for Suppressing Seedling Damping-Off Caused by *Pythium ultimum*

Steven J. Scheuerell and Walter F. Mahaffee

First author: Department of Botany and Plant Pathology, Oregon State University; and second author: U.S. Department of Agriculture–Agriculture Research Service-Horticulture Crops Research Laboratory, Corvallis, OR 97330.
Accepted for publication 14 June 2004.

ABSTRACT

Scheuerell, S. J., and Mahaffee, W. F. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. Phytopathology 94:1156-1163.

Compost tea is being used increasingly in agricultural production to control plant diseases. However, there has been limited investigation relating disease control efficacy to various compost tea production methods, particularly compost tea produced with active aeration and additives to increase microbial population densities in compost tea. Aerated compost tea (ACT) and nonaerated compost tea (NCT), produced with or without additives, was investigated for the suppression of damping-off of cucumber caused by *Pythium ultimum*. Compost tea was used to drench soilless container medium inoculated with *P. ultimum*; effect on damping-off ranged from not suppressive to consistently suppressive depending on the method used to produce the tea. The most consistent formulation for damping-off suppression was ACT produced with kelp and humic acid additives. Producing ACT with a molasses-based additive inconsistently suppressed damping-off; evidence suggests that residual nutrients can interfere with disease suppression. Heating or diluting compost tea negated suppression. Across all compost tea samples, there was no significant relationship of bacterial populations, measured as active cells, total cells, or CFU, to disease suppression. However, for all ACT produced without the molasses-based additive, there was a threshold of bacterial population density (6 $\log_{10}$ active cells per ml, 7.48 $\log_{10}$ total cells per ml, or 7 $\log_{10}$ CFU per ml) above which compost teas were suppressive.

Compost tea is being used increasingly as an alternative plant disease control measure in commercial horticulture (13). Compost tea is produced by mixing compost with water and incubating for a defined period, either actively aerating (aerated compost tea, ACT) or not (nonaerated compost tea, NCT) and with or without additives that are intended to increase microbial population densities during production (13,14). Compost tea applied to foliage has been demonstrated to suppress a range of foliar diseases (reviewed in literature citations 14 and 19); however, the use of compost tea as a soil drench for seed or root rot suppression has received very little attention.

The control of damping-off disease of seedlings, commonly caused by *Pythium* spp. in Northern latitudes, is of particular interest to greenhouse growers (15,16). Damping-off can be a severe problem when peat-based medium, which is naturally conducive to the pathogen, is used (5). The only investigation to date involving *Pythium* spp. and compost tea determined that pea seeds soaked in NCT, dried, and sown 2 days later had reduced disease symptoms on seedlings caused by *P. ultimum* (17). Heat treating the NCT negated all suppression of pathogen growth in vitro, indicating the likely role of the NCT microflora in disease suppression (17).

The microflora of both NCT (19) and ACT (6) are typically described as being dominated by bacteria, and therefore the bacterial population of compost tea could be a useful parameter to measure in relation to plant disease suppression. It has been proposed that increasing the population of total and active bacteria in ACT will generally increase the level of plant disease suppression (6). However, there is no published evidence supporting these assertions.

The objectives of this study were to determine if compost tea, produced from commercially available composts, applied as a drench suppresses seedling damping-off of cucumber caused by *P. ultimum* in a peat-based container medium that is naturally conducive to the disease; to examine how producing compost tea with aeration and additives impacts disease suppression; and to determine if the level of disease suppression is related to bacterial population density (measured as active cells, total cells, and colony forming units [CFU]) in compost teas.

MATERIALS AND METHODS

Producing compost teas. Three commercial sources of compost were used to make compost teas (Table 1). Yard trimmings compost (Rexius, Inc., Eugene, OR) was produced from ground landscape trimmings in windrows turned weekly for 3 months and then cured in a large pile for 9 months before sampling. One cubic yard of this material was cured an additional 2 years before being used for these experiments. Vermicompost was produced from mixed vegetation in a vertical flow reactor and marketed in 10-kg polyethylene bags (Soil Soup, Inc., Edmonds, WA). After receiving the bagged product, the compost was cured for 2 months in a shaded, open container. Tea compost is a proprietary blend of vegetative and animal manure-based composts sold for making compost tea (Rexius, Inc.). Approximately one cubic yard of the tea compost was cured for 5 months in a shaded, open container.

A Bio-blender (Soil Soup, Inc.) was used to produce ACT; mechanical aeration and liquid circulation was done by air injection through a submersed, hollow propeller shaft. Fifteen liters of tap water at 20 to 22°C was placed in a 19-liter bucket and aerated for 2 h to reduce chlorine present in the water. If used, additives were added to the water (Table 2). Compost inoculum was added to the liquid by immersing compost (500 g, approxi-

Corresponding author: W. F. Mahaffee
E-mail address: mahaffew@science.oregonstate.edu

Publication no. P-2004-0901-01R

mately 50% moisture, wt/wt) held in a 100-µm mesh filter bag (Soil Soup, Inc.). To assist the removal of soluble material and microorganisms from the compost, the filter bag was lifted above the water, allowed to drain into the bucket for 15 s, and then reimmersed for 30 s. This was performed a total of three times, with the filter bag left suspended in the liquid. The Bio-blender ran continuously for the remainder of the 36-h production cycle.

The NCT was started by adding 15 liters of tap water at 20 to 22°C to a 19-liter bucket, and the water was allowed to stand for 24 h for passive chlorine removal. If used, additives (Table 2) were added to the water followed by pouring 500 g of compost into the water. The entire contents were stirred vigorously for 20 s and then left undisturbed for 7 to 9 days until used.

Chemical and biological properties of compost and compost teas. *Chemical properties.* The pH and electrical conductivity (EC) were recorded twice for the vermicompost and tea compost and four times for the yard trimmings compost during the course of the study. Compost pH was determined from a saturated paste with a portable pH meter (Model 150; IQ Scientific Instruments, San Diego, CA) and EC was determined from a 2:1 (vol/vol) mixture of distilled water and compost with a portable EC meter (Model 933100; Hanna Instruments, Woonsocket, RI) (9). For compost teas, the pH, EC, temperature, and dissolved oxygen (Model 600; Engineered Systems & Design, Newark, DE) were recorded for each batch by immersing the probes into the bucket just before use in the *P. ultimum* bioassay.

Microbiological populations of compost. A 10-g sample of compost was added to 90 ml of sterile 0.02 M potassium phosphate buffer (PPB), pH 7.0, in a 250-ml shaker flask, and shaken (300 rpm, 25°C) for 20 min. The samples were serially diluted, plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) onto selective agar, and incubated at 22°C. Bacteria were enumerated on 5% trypticase soy broth agar (1.5 g of Difco trypticase soy broth and 15 g of agar per liter with 100 µg/ml cycloheximide [TSBAcyc^{100}]). Fungi were enumerated on water agar, pH 6 (18 g of agar per liter with 50 µg/ml rifampicin [WArif^{50}]). Yeast was enumerated on dilute, selective yeast media (SYM; 1.5 g of yeast extract, 2.5 g of peptone, 5 g of dextrose, 2.3 g of malt agar, and 17 g of agar per liter) amended with 100 µg/ml chloramphenicol, 50 µg/ml ampicillin, 500 µg/ml streptomycin sulfate, and 2 µg/ml dichloran. Population densities are reported as CFU/g of dry compost.

Microbiological populations of compost teas. A 1-ml sample of compost tea was aseptically removed from each bucket at the end of the culture period. Following serial dilution in sterile PPB, dilutions were plated on TSBAcyc^{100}, WArif^{50}, and SYM, and then incubated and counted as described previously. Populations were recorded as CFU/ml of compost tea.

Active and total bacterial cells in compost tea. Active and total bacterial cells were enumerated by epifluorescent microscopy and sequential digital imaging using the following procedure. Determination of metabolically active cells was done by staining with fluorescein diacetate (FDA; Sigma-Aldrich, St. Louis, MO), and determination of total cells was done with 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Stock solutions of DAPI (0.2 mg/ml DAPI in sterile deionized H_2O) and FDA (0.2 mg/ml

TABLE 2. Additives used to make compost tea

Additive recipe	Additive components per liter of water
No additive	None
Bacterial	5 ml of Bacterial Nutrient Solution (Soil Soup Inc., Edmonds, WA)
Fungal[z]	1.2 g of Maxicrop soluble seaweed powder (Maxicrop USA Inc., Arlington Heights, IL) 2.5 ml of Humax liquid humic acids (JH Biotech Inc., Ventura, CA) 3 g of rock dust (Target Glacial Dust; Target Products Ltd., Burnaby, B.C., Canada)

[z] Adapted from Ingham and Alms (7).

TABLE 1. Chemical and biological properties of compost and compost teas

Compost[n]	Compost tea aeration[o]	Compost tea additives[p]	*N*[q]	Dissolved oxygen[r] (ppm)	Temperature[r] (°C)	pH[r]	EC[r] (ds/m)	Population density (log_{10})[s]				
								Yeast[t] (CFU)	Filamentous fungi[u] (CFU)	Bacterial population		
										CFU[v]	Active cells[w]	Total cells[x]
Yard trimmings			4	...	...	5.7 ± 0.1[y]	0.87 ± 0.08	5.10 ± 0.11	5.25 ± 0.14	7.96 ± 0.21	...	...
	NCT	None	5	6.4 ± 0.5	15.5 ± 2.0	6.7 ± 0.3	0.26 ± 0.05	bdl[z]	0.69 ± 1.4	5.27 ± 0.26	5.31 ± 0.59	6.45 ± 0.42
	NCT	Bacterial	5	0.2 ± 0.1	15.2 ± 1.9	5.3 ± 1.0	0.95 ± 0.13	2.17 ± 2.5	3.84 ± 0.10	6.18 ± 0.51	5.76 ± 0.49	7.49 ± 0.32
	NCT	Fungal	5	0.2 ± 0.1	15.3 ± 2.3	6.9 ± 1.1	1.05 ± 0.08	0.73 ± 1.5	1.39 ± 1.6	7.14 ± 0.38	6.11 ± 0.39	7.26 ± 0.45
	ACT	None	6	8.5 ± 0.5	18.1 ± 1.6	7.4 ± 0.2	0.27 ± 0.03	bdl	0.70 ± 1.2	6.11 ± 0.41	5.84 ± 0.34	6.96 ± 0.40
	ACT	Bacterial	9	8.0 ± 0.4	18.5 ± 2.0	7.8 ± 0.5	0.96 ± 0.18	2.37 ± 1.6	0.58 ± 1.1	8.20 ± 0.38	6.98 ± 0.33	8.18 ± 0.36
	ACT	Fungal	13	8.2 ± 0.4	18.6 ± 2.6	8.6 ± 0.3	1.00 ± 0.11	bdl	1.05 ± 1.3	7.82 ± 0.22	6.87 ± 0.34	7.78 ± 0.47
Vermicompost			2	...	...	6.0 ± 0.8	4.70 ± 0.2	4.70 ± 0.02	6.09 ± 0.16	8.33 ± 0.33	...	...
	ACT	None	3	9.0 ± 0.6	17.8 ± 1.1	7.3 ± 0.5	0.77 ± 0.02	bdl	1.85 ± 1.6	6.02 ± 0.30	5.74 ± 0.06	7.20 ± 0.51
	ACT	Bacterial	4	8.6 ± 0.7	18.1 ± 1.1	8.0 ± 0.9	1.51 ± 0.23	2.86 ± 1.9	bdl	8.74 ± 0.16	7.35 ± 0.37	8.42 ± 0.28
	ACT	Fungal	4	8.7 ± 0.7	17.8 ± 1.9	8.5 ± 0.1	1.62 ± 0.18	1.50 ± 1.7	1.23 ± 1.4	7.84 ± 0.19	7.03 ± 0.25	8.11 ± 0.50
Tea compost			2	...	...	6.9 ± 0.2	3.52 ± 0.02	4.60 ± 0.64	6.32 ± 0.20	9.13 ± 0.16	...	...
	ACT	Bacterial	2	8.3 ± 0.6	17.3 ± 2.5	8.0 ± 0.4	1.93 ± 0.10	3.53 ± 0.21	2.10 ± 3.0	8.60 ± 0.28	6.92 ± 0.02	8.17 ± 0.24
	ACT	Fungal	2	8.2 ± 0.9	16.1 ± 1.0	8.5 ± 0.0	1.67 ± 0.02	bdl	1.97 ± 2.8	8.04 ± 0.49	6.86 ± 0.480	8.61 ± 0.50

[n] Yard trimmings compost (Rexius, Inc., Eugene, OR); vermicompost, vegetative-based vermicompost sold for compost tea (Soil Soup, Inc., Edmonds, WA); and tea compost, proprietary compost blend sold for compost tea use (Rexius, Inc.).
[o] ACT = aerated compost teas; NCT = nonaerated compost tea.
[p] Additives–none, bacterial, fungal, and bact-fungal fermentation additives described in Table 2.
[q] Number of batches.
[r] Recorded at end of ACT (36 h) and NCT (7 to 10 days) fermentation period.
[s] CFU/dry g for compost; CFU/ml for compost tea yeast, fungi, and CFU of bacteria; cells/ml for compost tea active cells and total cells.
[t] Colony forming units of yeast enumerated on dilute, selective yeast media (1.5 g of yeast extract, 2.5 g of peptone, 5 g of dextrose, 2.3 g of malt agar, and 17 g of agar per liter), amended, per milliliter, with 100 µg of chloramphenicol, 50 µg of ampicillin, 500 µg of streptomycin sulfate, and 2 µg of dichloran.
[u] Water agar (pH 6.0) with 100 ppm of rifampicin (CFU/dry g for compost; and CFU/ml for compost tea).
[v] Trypticase soy broth agar (5%) with 100 ppm of cycloheximide (CFU/dry g for compost; and CFU/ml for compost tea).
[w] Fluorescein diacetate stained after membrane filtration, cells per milliliter of compost tea.
[x] 4,6-Diamidino-2-phenylindole (DAPI) stained after membrane filtration, cells per milliliter of compost tea.
[y] Mean ± standard deviation.
[z] All samples below detection limit of log_{10} 2.3 CFU/ml of compost tea.

FDA in dimethyl sulfoxide) were kept frozen at –20°C with fresh stock used each day. A working solution of FDA was made by adding 1 ml of stock solution to 9 ml of PPB. For staining, an appropriate 10-ml suspension of compost tea was prepared from the serial dilutions used for plating on media. The DAPI stock solution (100 µl) was added to 10 ml of compost tea resulting in 0.002 mg of DAPI per ml. After 3 min incubation in darkness, the sample was vacuum-filtered through a black 0.2-µm filter (Isopore Membrane Filter 0.2-µm GTBP02500, 25-mm diameter, Millipore, Bedford, MA), on top of a support pad (MF Support Pad, AP1002500, 25-mm diameter, Millipore) in a 25-mm diameter glass microanalysis filter (#0-97563G, Fisher Scientific, Pittsburg, PA), which was covered with foil to reduce light exposure throughout the process. After filtration, the vacuum was stopped. Sterile PPB (1 ml) was gently overlaid on the filter, allowed to sit for 5 s, and vacuum filtered through the filter. The FDA working solution (1 ml) was overlaid on the filter, allowed to sit for 2 min, and then vacuum-filtered through the filter. The filter was immediately adhered to a glass slide with a 20-µl drop of sterile PPB and examined microscopically.

Digital imaging of stained cells was done at ×400 (Lieca DMRB Microscope, Heerbrugg, Switzerland) with 480/40-nm excitation and 510-nm LP barrier filters (GFP cube) and 360/40-nm excitation and 420-nm barrier filters (UV cube) to observe FDA- and DAPI-stained cells, respectively. The filter was viewed and a digital image was captured (Spot RT Slider diagnostic camera with Spot version 3.1 image capture software 2000 [Diagnostic Instruments, Inc., Sterling Heights, MI]) using the GFP cube, a second image was captured using the UV-cube. The stage was moved arbitrarily and the process was repeated until six pairs of images were obtained. Image acquisition was completed before visible quenching of the FDA-stained cells occurred.

Each pair of images were merged with the UV-cube image used as the source image and GFP-cube image as the active image, and semi-automated counting of the total number of stained cells was performed using Image Pro Plus version 4.1 software (Media Cybernetics, Silver Springs, MD). The counted cells were verified and manually recounted if necessary. The active cells, FDA stained (green), were manually counted from the merged image.

The process was repeated for each pair of images and the number of active and total cells were averaged across the six image pairs. To determine the number of cells per milliliter of compost tea, the average number of active and total cells from the six images was multiplied by 3,341.995973 to extrapolate the number of stained cells in the camera frame area to the exposed filter area (2.1 cm^2) and multiplied by the dilution factor.

Damping-off suppression by compost-amended container media or compost tea drench. *Compost-amended container media.* The three compost sources (described previously) were tested individually for their ability to suppress *P. ultimum* damping-off of cucumber when incorporated into container medium that was inoculated with *P. ultimum* and drenched with water. Three compost-amended container media were prepared by mixing compost (1:3, vol/vol) with commercial peat-perlite growing medium (Sunshine Mix #1, Sun Gro Horticulture, Inc., Vancouver, B.C., Canada).

Growing medium for compost tea. Compost tea drench treatments were tested for the ability to suppress *P. ultimum* damping-off of cucumber in 100% commercial peat-perlite growing medium (Sunshine Mix #1, Sun Gro Horticulture, Inc.) that was inoculated with *P. ultimum*.

Cucumber seedling assay. P. ultimum (isolated from corn roots, Willamette Valley, OR; provided by B. Hoinacki) inoculum was produced using 14-day cultures growing on Ko and Hora's (8) soil and chopped potato medium, dried, and sieved through a 1-mm^2 grid. Particles retained on a 0.25-mm^2 sieve were used to inoculate growing medium. For each compost-amended container medium or compost tea drench treatment, 2 liters of the respective growing medium was thoroughly mixed with *P. ultimum* inoculum (1.0 g/liter of growing medium) in a Twin Shell Dry Blender (Patterson-Kelley Co., East Straudsberg, PA) for 2 min. Inoculated medium was placed evenly into six 400-ml plastic nursery pots (experimental units). Eight cucumber seeds (*Cucumus sativus* cv. Marketmore 76) were sown 1 cm deep into each of the pots. For each compost tea drench treatment, compost tea was applied to the six replicate pots using a fine-spray watering can until the pots were saturated. Compost-amended container media were similarly moistened with tap water. The six pots for each treatment were placed, in random order, on separate nursery trays that served as experimental blocks. A large, clear, plastic bag was inflated and sealed around each nursery tray to simulate a germination room and maintain even moisture in the pots. The flats were placed in a 20°C growth chamber with a 16-h photoperiod. At 3 and 6 days after planting (DAP), each flat was vented to minimize changes in the atmosphere within the sealed flats. At 9 DAP, the number of healthy seedlings was recorded for each pot. A seedling was classified as healthy if it was growing normally and had no symptoms or signs of infection. Infection symptoms included a water-soaked or yellowing stem, wilted cotyledons, and stem lesion leading to seedling collapse; pathogen sign was white mycelia covering any portion of the seedling.

Effect of heating and diluting compost tea on disease development. The cucumber seedling assay was used to test whether heating or diluting compost tea impacted *Pythium* suppression. Compost tea was heated with continuous stirring to 95 to 98°C for 30 min and cooled to 25°C before applying to seeded pots as previously described. Diluted compost tea was prepared by mixing compost tea 1:1, 1:4, and 1:9 (vol/vol) with tap water before applying to seeded pots as previously described.

Effect of residual nutrients on disease development. The cucumber seedling assay was used to test whether the presence of excess nutrients from additives used to produce compost tea impacted *Pythium* suppression. Nutrient concentration in compost teas was manipulated in three separate ways. First, ACT was produced with 0.5, 1.0, and 1.5% bacterial additive (Table 2) with the assumption that not all of the available nutrients in the higher nutrient concentration treatments would have been used during the 36-h production period. Second, ACTs made with or without the fungal additive were amended with 0.1% (vol/vol) bacterial additive just prior to being used as a drench. Third, 0.01, 0.04, 0.125, and 0.3% molasses was added (vol/vol) to ACT made with the fungal additive immediately before drenching. The last two experiments simulated excess nutrients at quantified levels.

Experimental design and statistical analysis. All experiments were designed as randomized complete blocks with nursery flats as blocks and individual pots as the experimental unit. Each experiment had both pathogen-inoculated and noninoculated peat-perlite growing medium control treatments that were drenched with tap water. All experiments were repeated at least once.

Two-way analysis of variance was performed with treatment and block as factors; means were separated from the water drench control using mean separation tests such as Fisher's protected least significant difference, Duncan's multiple range, or paired *t* tests as indicated and appropriate. Linear regression was used to relate compost tea bacterial populations to the percent healthy cucumber seedlings. Percent healthy seedlings for each treatment were calculated by dividing the treatment mean healthy seedlings by the mean healthy seedlings of the noninoculated peat-perlite control treatment in each seedling assay. All statistical analyses were done with Statgraphics 4.0 software (Manugistics, Rockville, MD).

RESULTS

Chemical and biological properties of compost and compost teas. The pH, EC, and culturable populations of bacteria, yeast,

and filamentous fungi varied across the three compost sources (Table 1). In compost tea, the level of dissolved oxygen was influenced by the use of aeration and additives during production (Table 1). The average dissolved oxygen content across all ACT was 8.4 ppm. For compost tea made from the yard trimmings compost, the average dissolved oxygen content of NCT without additives was 6.4 ppm, significantly lower than 8.5 ppm for ACT made without additives ($P < 0.0001$). Adding either the bacterial or fungal additives to NCT decreased dissolved oxygen to 0.2 ppm. These NCT with greatly reduced oxygen conditions released highly putrescent odors upon drenching. Compost tea temperature was not influenced by production method; for all preparations, compost tea temperatures closely followed ambient temperatures (data not shown). Compost tea pH varied across the different compost tea production methods (Table 1). When ACT samples across compost sources were grouped by additive type, the average pH of compost tea made with no nutrient (pH 7.4), with bacterial additive (pH 7.9), and with fungal additive (pH 8.6) were significantly different from each other ($P < 0.0001$). The EC of all ACT produced without additives (0.40 ds/m) was significantly lower ($P < 0.0001$) than both ACT made with the bacterial additive (1.23 ds/m) and with the fungal additive (1.02 ds/m).

Microbial communities of both NCT and ACT were predominantly bacteria (Table 1), with most bacteria occurring as individual planktonic cells (data not shown). The average population of culturable bacteria, active bacterial cells, and total bacterial cells increased with the use of additives during compost tea production across compost sources (Table 1). While fungal populations were significantly lower than the source compost in all compost tea preparations, the highest culturable population densities of fungi were recovered from NCT produced with the bacterial additive (Table 1). A surface mat of organic material and microbial biomass partially consisting of sporulating filamentous fungi formed in these open, static buckets. However, there were no significant differences ($P = 0.46$) in the mean population of culturable fungi between the bacterial-additive and fungal-additive amended compost teas across all ACT samples (Table 1). In both NCT and ACT made without additives, the average culturable populations of yeast (CFU/ml) were at least 250-fold (vermicompost) and 629-fold (yard trimmings compost) lower than the source compost (CFU/dry g), while the average culturable fungi (CFU/ml) were 17,379-fold (vermicompost) to 36,291-fold (yard trimmings compost) lower than the source compost (CFU/dry g) (Table 1). Adding the bacterial additive consistently increased the average yeast population above levels found in compost teas made without additives (Table 1).

The bacterial population of compost tea measured as colony forming units, active cells, or total cells was influenced by the use of aeration and additives during production. Estimations of culturable populations were generally statistically equal or significantly greater ($P < 0.05$) than populations measured as active or total cells when additives were added to ACT (Table 3). For both ACT and NCT made without additives, the average bacterial culturable population was equivalent ($P > 0.1$) to the active cell population, while the total cell population was significantly greater ($P < 0.001$) than the culturable population (Table 3).

However, NCT made with either the bacterial or fungal additives had significantly ($P < 0.001$) greater total populations and significantly ($P < 0.01$) lower active populations compared with that of culturable populations (Table 3). With active aeration and addition of fungal additive, the total cell population was not different ($P > 0.1$) from the culturable population. With active aeration and bacterial additive, the culturable population was significantly greater ($P < 0.05$) than the total cell population (Table 3). Based on data for ACT produced with components of the fungal additive, adding kelp with or without the humic acids resulted in a similar relationship of bacterial colony forming units to active and total cells compared with the complete fungal additive recipe (kelp, humic acids, and rock dust) (Table 3).

Damping-off suppression by compost-amended container media or compost tea drench. When the yard trimmings compost, tea compost, or vermicompost was individually mixed (1:3, vol/vol) with the peat-perlite growing medium, damping-off caused by *P. ultimum* was significantly ($P < 0.05$) suppressed in one bioassay by the yard trimmings compost (Table 4). In contrast, these conducive composts were individually used to produce compost teas, that when used as a drench resulted in damping-off that ranged from not suppressive to consistently suppressive depending on the method used to produce compost tea. Severe damping-off was observed when NCT containing yard trimmings compost and no additive was used as a drench; disease severity was not significantly ($P > 0.05$) different from the inoculated control in five repeated bioassays (data not shown). Drenching with NCT produced with yard trimmings compost and either bacterial additive or fungal additive significantly ($P < 0.05$) suppressed damping-off in four of five and three of five repeated bioassays, respectively (data not shown).

Producing ACT without additives or with the bacterial additive resulted in inconsistent damping-off suppression over repeated bioassays (Table 4). When ACT was produced with a combination of bacterial and fungal additives, damping-off was not suppressed over three bioassays (data not shown). Producing ACT with any

TABLE 3. Comparison of methods to estimate bacterial populations in compost teas[s] made with or without aeration and additives

			Population density of bacteria ($\log_{10}$/ml)			Paired *t* test *P* value	
Aeration[t]	Additive components[u]	*N*[v]	CFU[w]	Active cells[x]	Total cells[y]	Bacterial CFU vs. active cells	Bacterial CFU vs. total cells
ACT	None	15	6.3 ± 0.42[z]	6.1 ± 0.37	7.2 ± 0.17	0.12	0.0002
ACT	Bacterial	20	8.5 ± 0.4	7.3 ± 0.23	8.3 ± 0.09	0.0001	0.005
ACT	Fungal (kelp, humic acids, rock dust)	24	7.9 ± 0.1	7.0 ± 0.16	8.0 ± 0.24	0.0001	0.23
ACT	Rock dust or humic acids	4	6.0 ± 0.29	5.7 ± 0.16	6.9 ± 0.19	0.16	0.02
ACT	Kelp or kelp and humic acids	4	7.7 ± 0.15	6.0 ± 0.3	7.5 ± 0.36	0.01	0.41
NCT	None	10	5.8 ± 0.71	5.8 ± 0.87	6.7 ± 0.57	0.69	0.0004
NCT	Bacterial	10	6.7 ± 0.47	6.0 ± 0.19	7.8 ± 0.51	0.01	0.0001
NCT	Fungal (kelp, humic acids, rock dust)	10	7.2 ± 0.4	6.4 ± 0.17	7.6 ± 0.26	0.0007	0.001

[s] All compost teas were made with yard trimmings compost, vermicompost, or tea compost; not all samples were used in the *Pythium ultimum* assays presented in this study.
[t] ACT = aerated compost tea; NCT = nonaerated compost tea.
[u] Fungal and bacterial additive components described in Table 2.
[v] Sample size.
[w] CFU/ml of compost tea; medium, 5% trypticase soy broth agar amended with 100 ppm of cycloheximide.
[x] Cells/ml; fluorescein diacetate stained after membrane filtration.
[y] Cells/ml; 4,6-diamidino-2-phenylindole (DAPI) stained after membrane filtration.
[z] Mean ± standard deviation.

compost and fungal additive alone suppressed damping-off in 19 of 19 *P. ultimum* bioassays (Table 4). When mixed with water and drenched, the additive formulations did not suppress damping-off (data not shown). Suppression of damping-off was not limited to peat-perlite growing medium. Suppression was observed with ACT produced with fungal additive in composted fir bark/peat-perlite growing medium (1:1, vol/vol) that was inoculated with *P. ultimum* (data not shown).

The bacterial population metrics of ACT were positively related to each other; linear regression of colony forming units and total cells ($P < 0.0001$; $R^2 = 0.67$) and linear regression of colony forming units and active cells ($P < 0.0001$; $R^2 = 0.77$) were significant. The relationship between percentage of healthy seedlings and bacterial population metrics of compost tea greatly depended on the types of compost tea that were considered. When all ACT were pooled, there was no linear relationship between the bacterial population metrics and plant health; linear regression analyses of the percent healthy seedlings of ACT against bacterial colony forming units, active cells, and total cells explained only 13.2, 7.07, and 7.77% of the variation, respectively. Likewise, when only ACT made with the bacterial additive was considered, based on regression analyses, there was no relationship ($P > 0.1$) between percentage of healthy seedlings and the bacterial population. However, when ACT made with the bacterial additive were excluded from the analysis, a significant ($P < 0.05$) positive linear relationship was observed between the bacterial populations and plant health (Fig. 1).

For the 18 NCT samples tested in bioassays (data not shown), no positive linear relationship existed between the average number of healthy seedlings and any bacterial population metric. Linear regression of healthy seedlings to bacterial colony forming units, total cells, and active cells had an R^2 of 0.13, 0.08, and 0.00001, respectively.

Effect of heating and diluting compost tea on disease development. Heating NCT or ACT to 95 to 98°C for 30 min and cooling to 25°C before drenching significantly ($P < 0.05$) increased damping-off compared with that of unheated compost tea (Table 5). Diluting compost tea 1:9 (vol/vol) with tap water significantly ($P < 0.05$) reduced disease suppression in five of six trials, with intermediate dilution rates having variable reductions in suppression (Table 5).

Effect of residual nutrients on disease development. When producing ACT, increasing the bacterial additive concentration from 0.5% to 1.0 or 1.5% (vol/vol) significantly ($P < 0.05$) decreased the number of healthy cucumber seedlings (Fig. 2), whereas mixing 0.1% (vol/vol) bacterial additive with suppressive ACT just before drenching negated disease suppression (Fig. 3). Since molasses constituted one-third of the bacterial additive by volume, the role of unused molasses in enhancing disease development was investigated. Mixing from 0.01 to 0.3% molasses with suppressive ACT just before drenching significantly ($P < 0.05$) reduced suppression (Fig. 4). In the absence of *P. ultimum*, the healthy seedlings resulting from drenching with either bacterial additive (0.5%) or molasses (0.3%) in water or compost tea was not different from drenching with 100% water (data not shown).

DISCUSSION

Drench application of compost tea can suppress *P. ultimum* damping-off of cucumber in soilless container media. Initial research on the use of compost teas for plant disease suppression utilized NCT that was generally produced without additives (19); however, recent attention has advocated the use of aeration and additives (6). In this study, both ACT and NCT significantly reduced disease; but consistent disease reduction was only attained with ACT produced with specific additives (i.e., kelp and humic acid). While NCT produced with yard trimmings compost and additives can suppress *P. ultimum* damping-off of cucumber, relatively long production times, and putrescent odors associated with NCT made with additives, detract from their utility. In addition, inconsistent disease control observed with NCT produced with yard trimmings compost and additives was observed also in preliminary bioassays using NCT produced with other compost sources (data not shown).

The most consistent formulation for damping-off suppression was ACT fermented with kelp, humic acids, and rock dust (termed fungal additive). The suppressiveness of this formulation was significantly reduced by heat treatment or dilution with tap water. Further characterization indicated that the rock dust component was not necessary for damping-off suppression and caused excessive wear on mechanical parts (data not shown). Suppression conferred with the fungal additive was independent of the compost source used in ACT production. This indicates that the selection of additives was more critical than the source of compost for producing ACT that suppressed *P. ultimum* damping-off.

TABLE 4. Capacity of compost-amended container media or aerated compost tea drench treatments to consistently suppress *Pythium ultimum* damping-off of cucumber seedlings in repeated bioassays

		Yard trimmings compost		Tea compost		Vermicompost	
Compost amendment[s] or Compost tea drench[t]	Compost tea additive[u]	Proportion of suppressive bioassays[v]	Healthy seedlings[w]	Proportion of suppressive bioassays[v]	Healthy seedlings[w]	Proportion of suppressive bioassays[v]	Healthy seedlings[w]
Compost incorporated 25% by volume[s]		1/3	2.44 ± 2.27	0/2	0.91 ± 0.35	0/3	2.50 ± 0.44
ACT[x]	None	1/6	2.31 ± 0.51	nd[y]	nd	1/3	3.28 ± 0.48
ACT	Bacterial	3/9	3.14 ± 1.53	0/2	2.92 ± 0.59	3/4	3.27 ± 1.99
ACT	Fungal	13/13	4.85 ± 0.77	2/2	5.25 ± 1.06	4/4	4.76 ± 0.97
P. ultimum-inoculated control[z]		0/13	1.43 ± 0.57	0/2	0.83 ± 0.71	0/4	1.46 ± 0.55
Uninoculated control		13/13	6.96 ± 0.53	2/2	6.75 ± 0.12	4/4	6.71 ± 0.34

[s] Yard trimmings compost (Rexius Inc., Eugene, OR); vermicompost = vegetative-based vermicompost sold for compost tea (Soil Soup, Inc., Edmonds, WA); and tea compost = proprietary compost blend sold for compost tea use (Rexius, Inc.). Compost mixed with peat-perlite growing medium (1:3, vol/vol; inoculated with *P. ultimum* and water drenched).

[t] Drenches applied to 100% peat-perlite growing medium inoculated with *P. ultimum*.

[u] Listed in Table 2.

[v] Proportion of significantly suppressive bioassays of total bioassays (significantly greater mean number of healthy seedlings than *P. ultimum*-inoculated control, according to least significant difference test, $P = 0.05$; determined separately for each bioassay).

[w] Mean number of healthy seedlings averaged over bioassays ± standard deviation. Treatments were applied to six replicate pots with eight seeds each sown in *P. ultimum*-inoculated peat-perlite growing medium. Mean number of healthy seedlings was determined by summing the number of healthy seedlings for each pot (0 to 8) and averaging these six values.

[x] ACT = aerated compost tea.

[y] Not determined.

[z] Peat-perlite growing medium inoculated with *P. ultimum* and drenched with water.

This has important practical implications for widespread production of disease-suppressive compost tea because additives are standardized materials, whereas the properties of composted materials can vary depending on fluctuations in feedstock materials, microbial decomposition dynamics, and environmental conditions.

Across all compost tea samples produced with or without additives, there was no significant relationship of bacterial populations, measured as active cells, total cells, and CFU, to disease suppression. However, when compost teas produced with the addition of molasses were removed, there was a threshold of bacterial population level above which compost teas were suppressive. The transition from nonsuppressive compost tea drenches to suppressive drenches occurred at approximately 6 $\log_{10}$ active bacterial cells per milliliter. The transition to suppressive samples as measured by total bacterial cells per milliliter is less dramatic. However, when samples with average healthy seedlings above 50% of the noninfested control were arbitrarily considered to be suppressive, then 15 of 16 samples with greater than 7.5 $\log_{10}$ total cells per milliliter would be considered suppressive. Using the same arbitrary definition of suppression, all compost teas that had greater than 7 $\log_{10}$ CFU per ml of bacteria would be considered suppressive. Therefore, it appears that ACT made without molasses-based additive can be divided into suppressive or nonsuppressive based upon threshold of bacterial populations as measured by active cells, total cells, and CFU (e.g., 7 $\log_{10}$ CFU/ml). Based on these data, monitoring bacterial populations could be a useful indicator for generating *Pythium*-suppressive compost teas and determining if suppressive compost tea could be diluted before use and still remain effective.

The use of aeration and additives in compost tea production appears to determine the proportion of the total bacterial population that was culturable. For NCT produced with or without additives, the population of total bacterial cells was significantly greater than the population determined by dilution plating, indicating the majority of cells would not grow under the culture conditions imposed. The same was true for ACT made without additives or when ACT made with rock dust or humic acids were considered as a group. Aggregation of bacterial cells could be a cause for lower culturable populations; however, extensive aggregation was not evident when observing samples for total and active cell enumeration (data not shown). For the above types of compost tea, the bacterial population, based on dilution plating, underestimates the total bacterial cell population and the dominant bacterial types might not be readily culturable.

However, in ACT made with the bacterial or fungal additives, bacterial populations as colony forming units were statistically equivalent or greater than the total bacterial cells. Having greater culturable populations than total populations is difficult to conceive. It is likely due to the observation that a slightly greater density of bacteria was localized at the outer edge of the stained filters (data not shown) and the image acquisition procedure did not account for this nonuniformity. Despite this phenomenon, it appears that either ACT made with the bacterial or fungal additives selectively increased culturable bacteria. These findings indicate that the simpler culturing procedure can be used to monitor the total bacterial population in ACT produced with readily available additives, and that it is possible to readily culture the dominant bacterial strains from these compost teas.

Since the ACT fermented with fungal additive had a pH of 8.5, the damping-off suppression observed in this study could have been due to reduced saprophytic activity of *P. ultimum* by increasing the pH of the growing medium. Increasing soil pH to 8.5 can cause increasing release of NH_4, which is known to suppress *Pythium* spp. activity (12). However, it is unlikely that disease suppression is primarily due to pH, since drenching with either the fungal additive in water (pH 8.6) or heat-treated fungal additive ACT (pH 8.5) resulted in the same disease incidence as drenching with water. In addition, all growing medium had a pH of 6.3 to 6.5 at the end of the 9-day cucumber bioassay (data not shown). Based on laboratory analysis of the well-cured yard trimmings compost, 13 ppm of NH_4 was present. Therefore, less than 1 ppm of NH_4 from the compost would be in solution after diluting approximately 25-fold (vol/vol) with water in compost tea production. Lastly, ACT made with a combination of the fungal and bacterial additives had a pH of 8.4 and did not suppress damping-off (data not shown). In total, these data support the conclusion that disease suppression afforded by compost tea applications is related to microflora present in the tea.

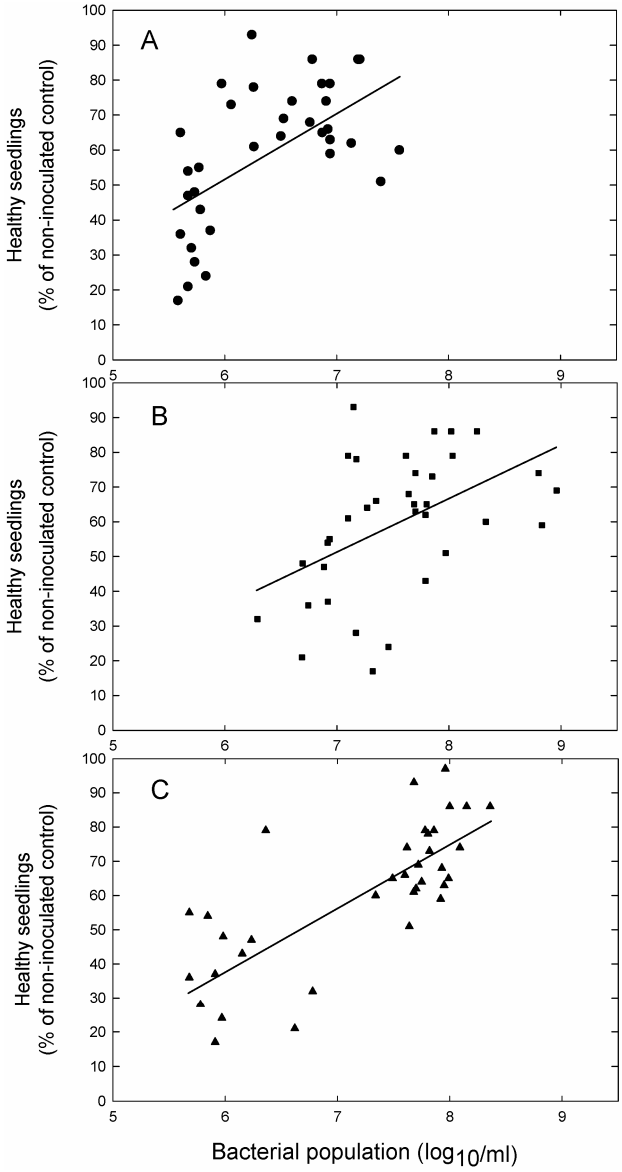

Fig. 1. Relationship of bacterial populations of aerated compost teas (ACT) to healthy cucumber seedlings in *Pythium ultimum* damping-off bioassays. For each compost tea drench treatment, healthy seedlings were scaled to percentage of noninoculated control (percent healthy seedlings = treatment mean healthy seedlings/noninoculated control mean healthy seedlings). The ACT with bacterial additive was not included. All ACT was produced either without additives or with single components, binary combinations of components, or all fungal additive components (0.12% [wt/vol] powdered soluble kelp, 0.25% [vol/vol] humic acids, and 0.30% [wt/vol] glacial rock dust). There was a significant linear relationship ($P < 0.05$ with $R^2 = 0.36$, 0.22, and 0.57 for **A, B, and C,** respectively. **A,** Active bacterial cells were measured by staining with fluorescein diacetate. **B,** Total bacterial cells were measured by staining with 4,6-diamidino-2-phenylindole (DAPI). **C,** Bacterial colony forming units were cultured on 5% trypticase soy broth agar amended with 100 ppm of cycloheximide.

TABLE 5. Effect of diluting and heating compost tea on seedling health

Compost[t]	Compost tea aeration[u]	Additives[v]	Dilution ratio[w] 1:0[x]	1:1	1:4	1:9	Heat-treated[y]
Yard trimmings	ACT	None	36.8 b[z]	...	...	...	15.8 a
Yard trimmings	ACT	Bacterial	51.2 b	...	...	...	0.0 a
Yard trimmings	ACT	Fungal	73.6 b	...	...	...	7.9 a
Yard trimmings	ACT	Fungal	61.5 b	...	...	...	20.5 a
Yard trimmings	ACT	Fungal	86.0 b	...	...	...	25.6 a
Yard trimmings	ACT	Fungal	63.6 b	34.1 a	31.8 a	25.0 a	...
Yard trimmings	ACT	Fungal	96.9 b	91.1 b	70.3 ab	44.2 a	...
Vermicompost	ACT	Fungal	74.3 cd	52.3 a–c	40.4 a–c	33.3 ab	19.0 a
Yard trimmings	NCT	Bacterial	70.4 b	...	...	15.1 a	...
Yard trimmings	NCT	Bacterial	85.3 b	...	...	10.4 a	18.3 a
Yard trimmings	NCT	Bacterial	86.9 b	...	...	...	39.5 a
Yard trimmings	NCT	Fungal	57.8 b	...	...	49.9 b	13.1 a

[t] Compost source described in Table 1.
[u] ACT = aerated compost tea; NCT = nonaerated compost tea.
[v] Additives described in Table 2.
[w] Compost tea was diluted with tap water at a given ratio.
[x] Compost tea was used without dilution or heat treatment directly from production bucket.
[y] Compost tea was heated to 95 to 98°C for 30 min and then cooled to 25°C for drenching.
[z] Healthy seedlings scaled to percentage of noninoculated control (% healthy seedlings = treatment mean healthy seedlings/noninoculated control mean healthy seedlings). Numbers within rows followed by the same letter are not significantly different ($P = 0.05$, Duncan's multiple range test).

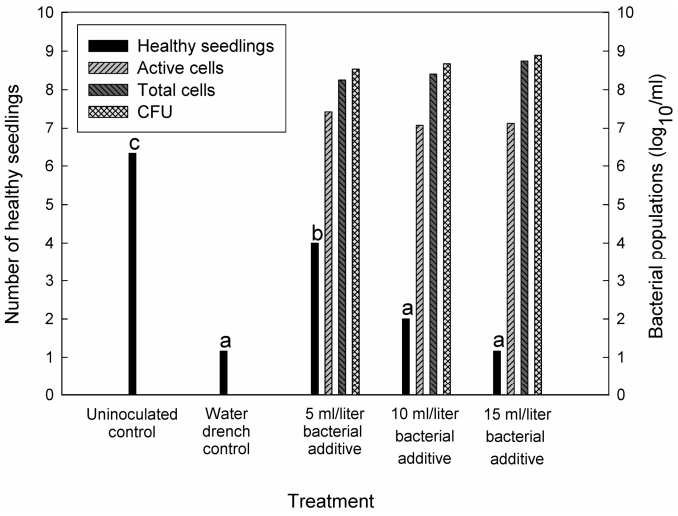

Fig. 2. Effect of molasses-based, bacterial additive concentration (Soil Soup, Inc., Edmonds, WA) used to make aerated compost tea on seedling health and bacterial populations. Active and total cells determined by direct counting after staining with fluorescein diacetate and DAPI, respectively. Populations as colony forming units were determined on 5% trypticase soy broth agar with 100 ppm of cycloheximide. Compost tea drench treatments were applied to six replicate pots each with eight cucumber seeds sown in *Pythium ultimum*-infested peat-perlite growing medium. Mean number of healthy seedlings was determined by summing the number of healthy seedlings for each pot (0 to 8) and averaging these six values. Letters labeling bars indicate significantly different number of healthy seedlings, and treatments with the same letter are not significantly different ($P = 0.05$, Duncan's multiple range test).

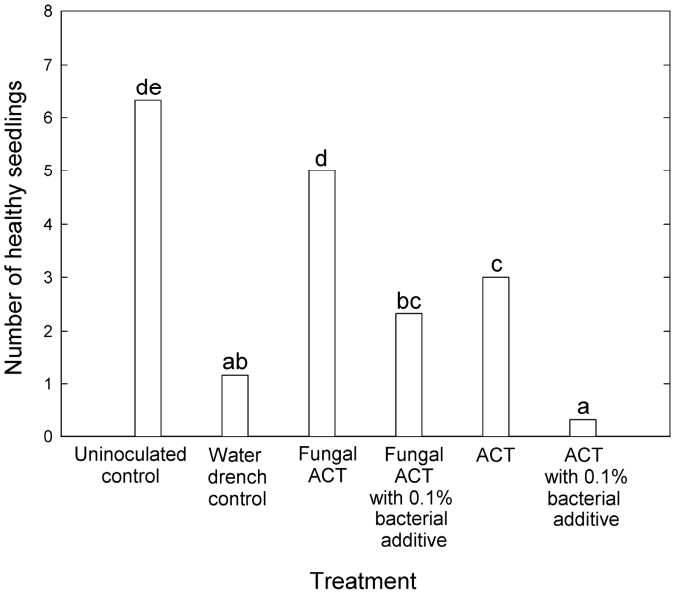

Fig. 3. Effect of tank mixing molasses-based Bacterial Nutrient Solution (Soil Soup, Inc., Edmonds, WA) with aerated compost tea (ACT) on seedling health. Compost tea drench treatments were applied to six replicate pots each with eight cucumber seeds sown in *Pythium ultimum*-infested peat-perlite growing medium. Mean number of healthy seedlings was determined by summing the number of healthy seedlings for each pot (0 to 8) and averaging these six values. Fungal ACT drench was produced with 0.12% (wt/vol) soluble kelp, 0.25% (vol/vol) humic acids, and 0.30% (wt/vol) glacial rock dust. Bars with the same letters are not significantly different ($P = 0.05$, Duncan's multiple range test).

There are several possible explanations why repeated experiments using ACT made with the molasses-based bacterial additive had erratic damping-off suppression. The most probable explanation is that residual nutrients, most likely sucrose, varied across compost tea batches and this enhanced *Pythium* propagule germination and growth or reduced competition for seed exudates, resulting in enhanced pathogen growth and infection. It is generally accepted that bacteria can indirectly protect seeds against *Pythium* infection by metabolizing seed-exudate stimulants (18) and that competition for available nutrients is a likely mechanism of soilborne pathogen suppression if adding nutrient amendments negates suppression (10). Evidence supporting this conclusion includes the inverse relationship between the concentration of bacterial additive used in ACT production and disease suppression; ACT produced with increasing concentrations of sucrose as the sole fermentation nutrient corresponded to increased damping-off disease levels (data not shown); and the addition of as little as 0.01% molasses or 0.1% bacterial additive to suppressive ACT significantly increased damping-off. Similarly, suppression of cucumber seedling damping-off caused by *P. ultimum* in compost-amended container medium was negated by adding 0.375% sucrose and 0.075% asparagine to the growing medium (3).

Residual nutrients could also suppress antibiotic production or parasitic activity in favor of saprophytic metabolism. This would affect bacteria capable of producing antimicrobial compounds that reduce *Pythium* germination through fungistatic or fungicidal effects (18). Excessive nutrients have been shown to reduce hyphal lysis of *P. aphanidermatum* by antagonistic bacteria in separated

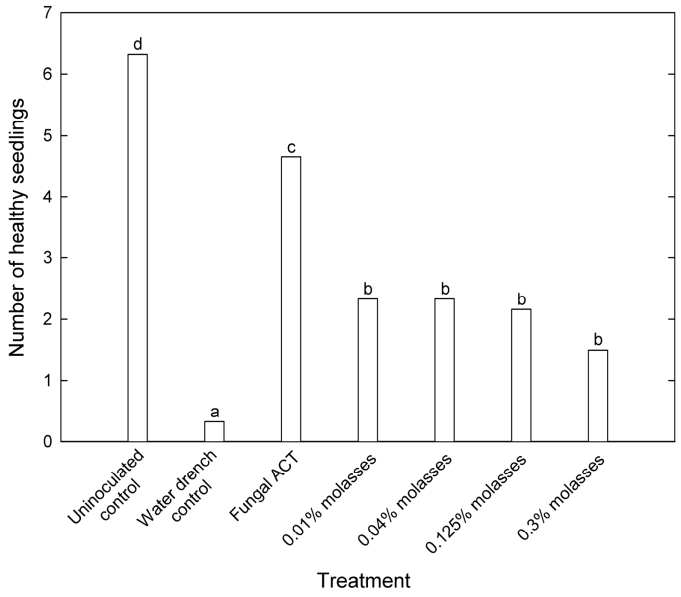

Fig. 4. Effect of tank mixing molasses with aerated compost tea (ACT) on seedling health. Compost tea drench treatments were applied to six replicate pots each with eight cucumber seeds sown in *Pythium ultimum*-infested peat-perlite growing medium. Mean number of healthy seedlings was determined by summing the number of healthy seedlings for each pot (0 to 8) and averaging these six values. The fungal ACT drench was produced with 0.12% (wt/vol) soluble kelp, 0.25% (vol/vol) humic acids, and 0.30% (wt/vol) glacial rock dust. Bars with the same letters are not significantly different ($P = 0.05$, Duncan's multiple range test).

cattle manure-compost medium (11). Amending the container medium with a glucose-asparagine mixture (3.36:1, wt/wt), amended at 0.5% wet weight, reduced hyphal lysis to 18% over a 24-h period compared with 80% lysis for nonamended medium (11). Additionally, drenching the glucose-asparagine mixture (1% solution in water) onto the compost medium negated cucumber damping-off suppression (11).

While further work is needed to directly quantify the residual sucrose concentrations in ACT produced with molasses-based additive to determine the effect on damping-off suppression, there are strong indications that the use of simple sugars as additives should be avoided when producing compost tea for disease suppression. In addition to the potential of residual nutrients increasing Pythium damping-off, the use of simple sugars in producing compost tea has been linked to growth of *E. coli* in aerated compost tea makers when compost contaminated with *E. coli* was used (1). Other work determined that *Salmonella enterica* and *E. coli* O157:H7 did not grow when inoculated into nonaerated flasks containing 20 g of compost and 180 ml of sterile water; however, incremental additions of molasses were related to increasing growth of both organisms (4). Other unpublished work suggests that using additives with compost that is free of human pathogens does not pose a safety risk (13). However, commercial testing of compost for human pathogens cannot guarantee a pathogen-free product due to potential sampling errors and detection limits of common analytical procedures (13). Further work is needed to characterize additives that sufficiently increase microbial populations for plant disease control yet do not increase human or plant pathogens (13).

An increased understanding of the quantitative relationship between compost tea microbial populations and plant disease development could help develop guidelines for producing suppressive drench formulations, similar to guidelines for compost-amended container medium that prescribe minimum levels of microbial activity and biomass to prevent *P. ultimum* damping-off (2). Information on the duration of suppression afforded by drenching container medium with compost tea would be useful to develop an application schedule for long-term suppression of Pythium damping-off in commercial production. Further experimentation on a production scale is needed to develop this method into an effective disease control tool.

ACKNOWLEDGMENTS

We acknowledge Soil Soup, Inc., for supplying Bio-Blenders for producing aerated compost tea. We thank B. Short for technical assistance and B. Hoinacki for the *P. ultimum* culture. Funding for this research was provided by USDA CRIS 303-5358-22000-024-00D and the Oregon Association of Nurseries and Oregon Department of Agriculture.

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